Remarks

I. The new claims

The new claims are patterned generally on those that are already pending herein. The Examiner should note modification of the language in Claim 200 (compared to Claim 164) and in Claim 201 (compared with Claim 172) to indicate that stabilization of CFTR-encoding sequence within a DNA molecule that contains it can be accomplished by a modication in the DNA molecule that is outside of the CFTR-encoding sequence itself. This situation arises, for example, when the DNA molecule is itself a plasmid and a regulatory element thereof (for example, the origin of replication) is chosen so that the plasmid will be maintained in a host E. coli cell only at low copy number.

Claims 200 and 201 also permit (as, for example, does Claim 164) that the CFTR-encoding nucleotide sequence that is being stabilized be other than a cDNA (for example, a partial genomic "minigene" sequence), as long as the cryptic promoter sequence that otherwise causes inviability of host E. coli cells is present therein.

That Claim 199 (directed to use of only a single point mutation to inactivate the cryptic promoter) is patentable over the prior art is further established by Dr. Alan Smith's Rule 1.131 Declaration submitted herewith.

II. Rejections previously made of record

The Examiner has indicated (see page 2 of the Action of December 9, 1994) that all objections and rejections concerning the patentability of the Claims that were pending prior to the date of that Action have been withdrawn. Given the extensive history of the application, and the existence of three parent applications

thereof, 07/613,592; 07/589, 295; and 07/488,307, Applicant's would like to briefly address certain issues pertaining to the prior rejections in order to confirm that they have been overcome, or are no longer considered relevant.

(A) Please refer to the Examiner's Action of March 24, 1992 (copy attached) in the 07/488,307 parent application. At numbered paragraph 28 of the Action, the Examiner noted that Riordan et al 1989 (Document B in the Exhibit Book) did not disclose isolation of the CFTR-encoding cDNA. Nonetheless, the Examiner took the position that assembly of such a cDNA was not patentable over the Riordan et al. 1989 disclosure because once a depiction of the predicted sequence was published, such a cDNA could have been constructed by a synthesizer machine. [Presumably it might also be suggested that if the population of molecules so synthesized was very small, then it could be amplified by PCR technology.]

In response, Applicants invite the Examiner to consider the limitations — **especially as existed in 1990** - inherent in PCR or synthesizer technology. If the Examiner wishes to maintain this rejection, citation to the record of appropriate references showing the successful application of either or both of the above-mentioned techniques to construction of a 4.5 kb polynucleotide is requested.

It is also noted that since these techniques introduce errors into the nucleotide sequence of the polynucleotides so generated, there would result a population of very similar DNA molecules, but in which the individual polymers differ from each other in a multitude of minor — but unpredictable — ways. It is believed that isolating the wild type cDNA from such a mixture in purified form would require a further procedure such as cloning — which cloning would still

be subject (absent reference to Applicants' novel teachings) to the very same difficulties that prevent stable propagation in bacteria. It is believed that Applicants' use of the term "purified" and/or the term "isolated" in their claims properly distinguish the discoveries defined thereby.

(B) Please refer to the Examiner's Final Action of January 21, 1993 (copy attached) in the 07/488,307 parent application. In the first complete paragraph of page 4 thereof, the Examiner refers to the low copy number strategy for stabilizing propagation of CFTR-encoding cDNA in host E. coli cells as "old and well known". Applicants respectfully observe that this simply uses Applicants' own teachings as a reference against the very application that provides them. Applicants believe that this issue has been addressed (see pages 15-16 in Applicants' Reply herein of June 9, 1995). The Examiner may also have confused the problem of preventing over-production of an encoded protein when production thereof was nonetheless intended in the host bacteria (see the Harris and Carrier references that were cited in the Official Action) with the problem of understanding (and then overcoming) why the CFTR-encoding cDNA could not be isolated as a full length clone, nor stably propagated in bacteria, when no expression of CFTR protein therein was ever intended. Additionally, Applicants herein have cited to the record a publication of M. Drumm et al. (see Document H in the Exhibit Book of June 9, 1995) that specifically teaches away from the use of low copy number methodology to stabilize propagation of CFTR-encoding cDNA in host bacterial cells.

(C) Please refer to the Examiner's Final Action of January 21, 1993 (copy attached) in the 07/488,307 parent application. At the fifth complete paragraph of page 2 thereof, the Examiner refers to U. S. Patent No. 4,322,274 issued to G. B. Wilson et al. in 1982 which refers to "the cystic fibrosis protein" (G.B. Wilson should not be confused with James M. Wilson, co-inventor on U.S. Patent 5,.240,846, issued August 31, 1993, which pertains to CFTR protein, and the gene therefor). Applicants believe that since reference to the 1982 "discoveries" of G.B. Wilson et al. appears to be absent from the recent scientific literature, such "discoveries" , whatever they may involve, may be considered unrelated to CFTR protein, and its encoding DNA. Citation to the record of any approporiate reference to the contrary would be appreciated.

(D) Please refer to the Examiner's Action in the present application mailed November 23, 1993. At page 9, line 13 thereof, and then on to the end of the page, the Examiner is referring to the teachings of U. S. Patent 5,240, 846 issued to Collins et al. Applicants reiterate that there is no disclosure in any of the original parent applications filed in 1989 , and to which the Collins et al. patent claims priority under 35 USC § 120, that describes successful cloning of a DNA encoding full length CFTR protein, or that describes expression from a full length cDNA of functional CFTR protein in host cells. Such disclosure, where it appears in the '846 patent, is entitled only to the **September 18, 1990** filing date of the 07/584,275 parent application. Applicants herein have taken all necessary steps to swear behind the September 18, 1990 date. In this regard, see Applicants' Reply with Amendment of June 9, 1995 (at pages 22-23 thereof) and Declarations " C" and "D" attached thereto, and also Declarations "E" and "F" submitted herewith.

III. Reduction to practice of inventions involving the 936 T to C mutation

As stated in the Reply with Amendment submitted herein on June 9, 1995 (see, for example, page 20 thereof, and also paragraph 4 of Dr. Gregory's acccompanying Declaration "D"), Applicants believe that the publication Gregory et al. "Expression and Characterization of the Cystic Fibrosis Transmembrane Conductance Regulator", Nature, 347, issue of September 27, 1990, pp. 382-386 represents a complete reduction to practice of the invention that provides for stable propagation in E. coli of CFTR-encoding cDNA, achieved by selection of one or more point mutations that will inactivate the cryptic promoter in the cDNA.

Simply stated, Figure 1 of the Gregory et al. publication places the scientific community in complete possession of this invention in that the location of the promoter-capable sequence in the cDNA has been correctly determined. That the 936 "T" nucleotide of CFTR cDNA corresponds to the highly conserved 3' terminal "T" nucleotide of the E. coli consensus (see page 9 of the reference W.S. Reznikoff and W.R. McClure, "E. coli Promoters" in W. Reznikoff et al. eds., Maximizing Gene Expression , Butterworths Publishers, Boston, MA, 1986, at Chapter 1, pp. 1-34, provided herewith in the Supplemental Information Disclosure Statement), is provided for at page 382, column 1 of the Nature paper.

As the Examiner will recognize, it is but ministerial to verify that nucleotide 936 (see Riordan et al. 1989) is the last nucleotide in an AAT Asn codon, and that mutation thereof to AAC not only alters the most highly conserved nucleotide in the consensus (see the W.S. Reznikoff and W.R. McClure reference), but also provides the only way to preserve encoding of Asn at this

position. Thus, although literal mention of the 936 T to C mutation is not made in the Gregory et al. publication, Applicants assert that any person remotely familiar with the art has been placed completely in possession of this important invention. Should the Examiner consider that the Nature paper of Gregory et al. is somehow less than a complete reduction to practice of this important invention (i.e. the cDNA stabilized by one or more point mutations), then the Examiner's attention is respectfully directed to the attached Declaration "F" of Dr. Richard Gregory and its Exhibits 1 and 2.

Exhibit 1 is copy of a page from Dr. Gregory's laboratory notebooks at the Genzyme Corporation and dated prior to July 27, 1990, the date that the manuscript for the Nature paper was received for publication by that journal. The notebook page clearly shows that a mutation selected to facilitate propagation in E. coli of the CFTR cDNA is T936C, as further evidenced by depiction of an appropriate oligonucleotide for site directed mutagenesis of the CFTR-encoding sequence.

Exhibit 2 is a further page from Dr. Gregory's laboratory notebooks that was dated prior to September 18, 1990. September 18, 1990 is the earliest effective date of any reference against the present application that discloses stable propagation of full length CFTR-encoding DNA (In this regard, see the text of Declaration "F"; section (II)(D) of this Supplemental Reply; and pages 22-23 in the Reply with Amedment submitted herein on June 9, 1995).

Exhibit 2 records successful placement, prior to September 18, 1990, of a CFTR-encoding cDNA containing the T936C mutation into a plasmid. The CFTR nucleotide sequence included therein extends from the codon for the CFTR initiator methionine (nucleotide 133) to well past the codon for the C-terminal amino acid thereof.

IV. Oral remarks of Dr. Lap-Chee Tsui made in April and June of 1990

Attached to this Supplementary Reply is a Declaration under 37 CFR 1.131 of Dr. Alan E. Smith, of the Genzyme Corporation, Assignee of Record herein. The Declaration is provided in order to bring to the Examiner's attention the subject matter of certain oral remarks made by Dr. Lap-Chee Tsui, a co-inventor/co-author of many of the references that have been cited against the present Applicants, at conferences on cystic fibrosis held in April and in June of 1990. The remarks pertained to his then-ongoing attempts to provide a full length CFTR-encoding cDNA.

Dr. Smith's Declaration is provided in order to demonstrates that Dr. Tsui's remarks were conflicting and unclear, and did not place the art-skilled audiences for these remarks in possession of any inventions pertaining to provision of the full length cDNA. That this is so is demonstrated, in part, by making reference to a British patent application of Tsui et al. filed at a later date, in September of 1990, that makes clear the serious technical mistakes that still affected the "inventions" that may have been described orally in April and June of 1990.

Dr. Smith's Declaration is believed to be self-explanatory when studied in connection with the items provided in the attached Information Disclosure Statement. The Declaration is organized as follows:

(A) description of inventions (and the timeframe thereof) made by Genzyme inventors relating to stabilization of the CFTR- encoding cDNA against the effects of the cryptic bacterial RNA polymerase promoter.

(B) discussion of the scientific mistakes in, and confusing nature of, the British patent application of Dr. Tsui et al. entitled "Stable Propagation of Modified Full Length Cystic Fibrosis Transmembrane Conductance Regulator Protein cDNA in Heterologous Systems" (which relates to the making of point mutations in CFTR-encoding cDNA), as filed on **September 21, 1990**; and

(C) discussion of certain oral remarks made by Dr. Lap-Chee Tsui at conferences held in **April and June of 1990** (preliminary to the September 21, 1990 British filing) that concern the making of point mutations in CFTR-encoding cDNA.

Briefly, the Declaration demonstrates that Dr. Tsui's attempts to identify nucleotide sequence changes useful to stabilize the CFTR-encoding cDNA for propagation in E.coli were affected by an apparent lack of understanding that a cryptic promoter was responsible for the cloning difficulties. Mutations that Dr. Tsui described as "effective" are believed to be inoperative, and were apparently selected either by (1) reliance on an incorrect explanation as to why CFTR-cDNA is unstable in E. coli, or (2) from the later failure of Tsui et al. to correctly identify the location of the cryptic promoter. As the Declaration also provides, the highly effective T936C mutation was considered inoperative by Tsui et al.

Conclusion

No fees are believed to be due for the new claims since both the number of independent claims and the number of total claims that are added hereinby are less than the numbers thereof that have been canceled previously. No Request for an Extension of Time is believed to be due in connection with this Reply since it is a supplemental reply, and since an appropriate request was timely made in connection with Applicants' submission of June 9, 1995. However, should the Patent Office determine that any fees are due, then the Patent Office is authorized to charge to Deposit Account **07-1074** the additional claim fee, or any other fee, that it determines is necessary to secure the filing of this Amendment or entry of the new claims.

Applicants firmly believe that the application is fully in condition for allowance, and that all pertinent issues have been addressed. It is respectfully requested that the claims now be passed to allowance. The Examiner is invited to phone the undersigned at (508) 872-8400 to discuss any matters that she believes require further attention. It is further suggested that a telephonic Interview may help to resolve remaining issues. Applicants herein would again like to acknowledge the Examiner's efforts on behalf of the application, and the medically important discoveries that are described therein. An early and favorable action is respectfully requested.

Respectfully submitted,

GENZYME CORPORATION

Dated: 8/18/95

By: William G. Gosz

One Mountain Road
Framingham, MA 01701
(508) 872-8400

William G. Gosz,
Reg. No. 27,787
Attorney for Applicants